The Use of Laser Scanning Confocal Microscopy in Detecting Bone Microstructure Using Basic Fuchsin and Toluidine Blue Stains

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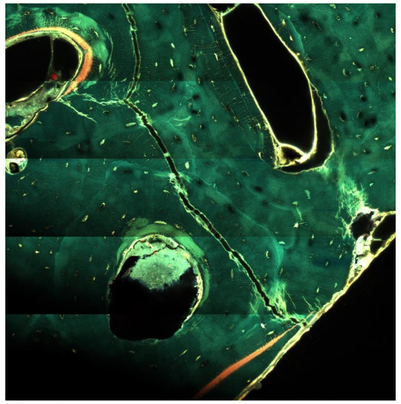

Fig. 1

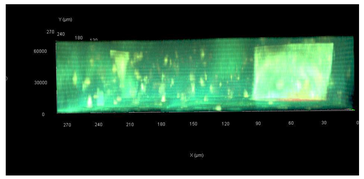

Fig. 2

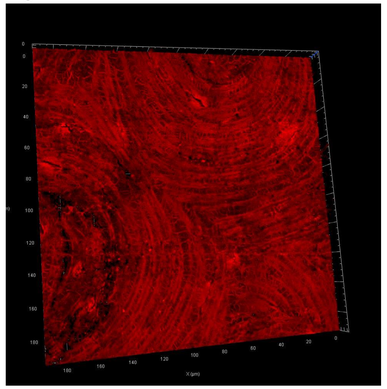

Fig. 3

Introduction

3D analysis of bone is nothing new to osteology. Whether it be the skull to the femur, anthropologists have been using tools to develop 3D models of bones for a variety of reasons. On the microscopic vision, anthropologists have been using technologies such as microCT to SEM to get a greater understanding of bone on a histological level. The purpose of this pilot project is to determine the usefulness of laser scanning confocal microscopy on thin sections of bone using a basic fuchsin stain and toluidine blue to determine its usefulness in assessing the microstructure on bone. Laser scanning confocal microscopy uses laser light set at specific excitation wavelengths to generate targeted emission wavelengths. These wavelengths are the projected via there intensity at specific pixels to generate an image. Using this technology, the purpose of this project is to gain a better understanding of the lamellar interaction on a microscopic level.

Methodology

This project utilized 3 femoral cross sections with 1 unstained control 2 stained with basic fuchsin stain and 3 femoral samples stained with toluidine blue. The stain was applied following the protocol as established by the manufacturer, Sigma Aldrich. All specimens were examined using a Carl Zeiss™ LSM 800 laser scanning confocal microscope. Tile images of 5 x 5 were used with a z-stack of 71 images for a total of 1,775 images per sample. Each sample was scanned at 2586 x 2586 pixels per image and a scanning speed of 9. All lasers except the 555nm wavelength were turned off in order to visualise just the mineral content of the bone for the basic fuchsin stain. The toluidine blue samples were imaged using 555 nm and 648nm lasers. For the unstained bone, all lasers were turn on (405nm, 488nm, 555nm, 648nm) though the 488nm and 405nm emissions were the most prevalent.

Results

The results of this study found that the use of basic fuchsin stain when magnified using a laser scanning confocal microscope the mineral sub-structure of bone can be visualised. In particular, I was able to view the relationship between the lamellar spaces. The results of this project found that in the circumferential lamellar bone along the periosteum, there is limited space and the bands are quite compact (Fig. 3). When I moved more to the center of the compact bone, we see larger gaps between the lamellar spaces (Fig. 3 & 4). We additionally see sections of bone in the extra osteonal space, or primary osteons, where osteocytes are found (Fig. 3). In addition, when visualizing the toluidine blue samples, we can further visualize the lamellar banding (Fig. 5 & 6). Further, we can tell more about edge geometry on damaged bone (Fig. 5 & 6). Current studies will help determine if such damage is perimortem or postmortem in nature. Overall, this project found that laser scanning confocal microscopy when examining a bone stained with basic fuchsin is a good tool in examining the 3D microstructure of bone on a histological level.

Discussion and Conclusion

There are many tools that can be used to ascertain the mineral make-up of bone. From SEM to microCT, we can develop bone in a 3D model like never before. The results of this project have found that laser scanning confocal microscopy is one of those tools and can be a valuable way of examining the microstructure of bone on a histological level. Further, by knowing this, we can use other techniques to differentially stain the non-mineral portions of bone, deselect the 555nm wavelength and then image the organic over the inorganic. This step would be useful when examining the collagenous portions of bone or other proteins and bone cells. Overall, laser scanning confocal microscopy has demonstrated a usefulness in examining histological samples of bone which other modalities may miss.

Special thanks go out to Dr. Tracy Rogers, and the UTM Department of Biology for their assistance and use of the confocal microscope

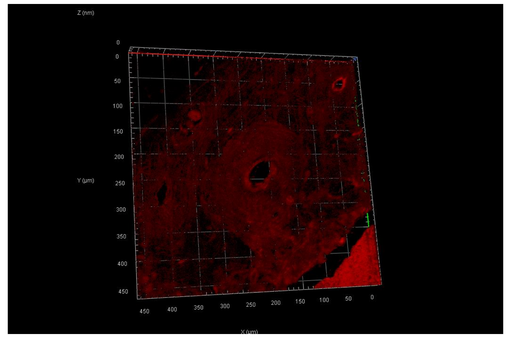

Fig. 4

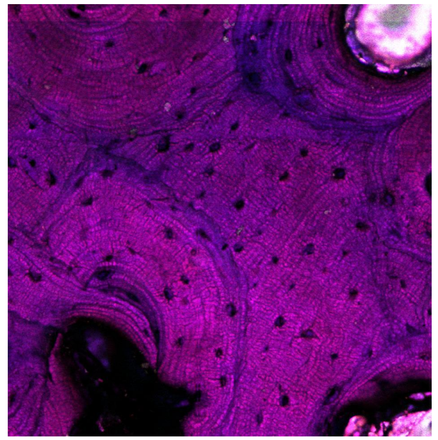

Fig. 5

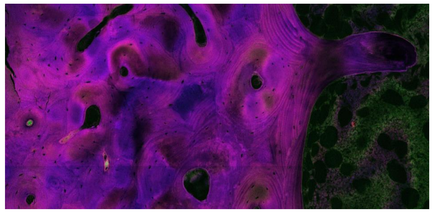

Fig. 6